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14. ABSTRACT

Our goal in year 2 was to use the tissue slice (TSC) NMR compatible bioreactor optimized in year 1 to identify hyperpolarized metabolic biomarkers of prostate cancer presence and aggressiveness. To accomplish this goal my group finished the engineering of a 5 mm bioreactor and acquired hyperpolarized [1-13C]pyruvate data indicating that similar signal to noise and quality data can be achieved with 4 to 5 prostate tissue slices in the 5 mm bioreactor as was acquired from 30-40 tissue slices in the prior 10 mm bioreactor. The time course of viability of the tissue slices prior to and after the bioreactor 5 mm bioreactor study was also established using a live-dead tissue assays performed by the co-PI's Peehl and Ronen on this proposal. We subsequently utilized this optimized 5 mm bioreactor in hyperpolarized [1-13C] MR studies to begin correlating hyperpolarized [1-13C] pyruvate metabolism with pathologic grade (aim 2) and observed a grade dependent increase in the flux of hyperpolarized [1-13C]pyruvate to [1-13C]lactate, with higher levels of lactate in higher pathologic grade cancers. More cancer TSCs need to be studied to demonstrate biologic significance.

15. SUBJECT TERMS
Prostate cancer, imaging, tissue model

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INTRODUCTION

The overall objectives of this synergistic research is to optimize an MRS-compatible, 3D Tissue Culture Bioreactor for use with primary human prostate tissue cultures (TSCs) and use it to identify hyperpolarized molecular imaging biomarkers for improved prostate cancer patient-specific treatment planning and early assessment of response to hormone and chemotherapy. The hypotheses that will be tested in this project are that fresh human prostate tissue slices can be maintained without loss of structure, function or metabolism within a NMR compatible 3-D tissue culture bioreactor, and that magnetic resonance spectroscopy studies of these human tissue slices can be used to identify hyperpolarized metabolic biomarkers of prostate cancer presence and aggressiveness and early response to therapy. The goals of this study will be achieved through the following specific aims. The first aim is to optimize conditions for maintaining human prostate tissue slices (TSCs) in an NMR- compatible, 3-D tissue culture bioreactor and to verify the metabolic integrity of TSCs over time. The second aim is to use this new experimental model to compare the metabolism of normal and malignant prostate tissues, and importantly, determine whether hyperpolarized metabolites correlate with pathologic grade (i.e., Gleason grade 3 versus grade 4/5). The third aim is to use NMR to evaluate the response of TSCs to anti-androgen therapy and chemotherapy. In the final report below, I summarize the contributions of my lab to this synergistic project which has culminated in a recently published manuscript entitled "Metabolic reprogramming and validation of hyperpolarized 13C-lactate as a prostate cancer biomarker using a human prostate tissue slice culture bioreactor", a submitted manuscript entitled "Optimization and comprehensive characterization of a faithful tissue culture model of the normal and malignant human prostate". We believe that this clinically relevant metabolic

model system in combination with HP MR can facilitate the identification of clinically translatable biomarkers of prostate cancer presence, aggressiveness, and treatment response in future studies.

BODY

The first Aim was to optimize and validate conditions for maintaining the structure and function of TSCs in an MRS-compatible, 3-D tissue culture bioreactor. As described in the previous years progress reports, my group's research focused on the engineering and testing of a NMR compatible bioreactors that allow the study of living human prostate tissue slices within high field NMR spectrometers. To accomplish this goal, we have engineered and optimized a novel MR-compatible bioreactors (**Fig. 1**) that can maintain metabolically viable human TSCs and have performed bioreactor ^{1}H MRI/^{31}P and dynamic ^{13}C spectroscopy studies within high field NMR spectrometers coupled to a Hypersense DNP polarizer. We have recently published (Keshari et al., Prostate, 2013, see "Reportable Outcomes" section) on the use of this NMR compatible bioreactor to metabolically characterize living benign and malignant human prostate tissue slices obtained at surgery and demonstrated that they accurately recapitulate features of benign and malignant prostate tissues in vivo (**aim 2**). As indicated in Dr. Peehls final report,

her lab has also improved the longevity of the TSCs in standard culture conditions, extending the maintenance of structure and function up to 5 days (Maun et al, submitted to Lab Invest., see "Reportable Outcomes" section. More recently, we have also demonstrated in conjunction with Dr. Ronen that human prostate TSC's can also be maintained for up to 5 days in our MR compatible bioreactor, and TSC viability is reduced when androgen levels are reduced to castrate levels.
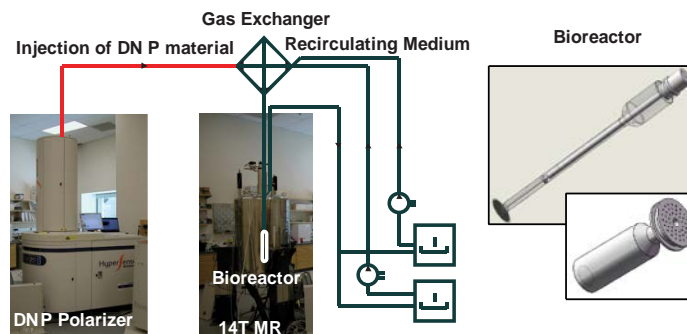

Figure 1. Schematic of a bioreactor (3-D culture perfusion system with a continuous flow of 37ºC oxygenated medium to mimic physiological environment), and its interface with HP MR.

Aim 2 was to use the TSC/NMR bioreactor to identify hyperpolarized metabolic biomarkers of prostate cancer presence and aggressiveness. As described in Keshari et al., Prostate 2013, we investigated the metabolic reprogramming that occurs with the evolution and progression of prostate cancer using the optimized bioreactor described in aim. Specifically, the steady-state metabolite concentrations in freshly excised human prostate TSCs provided by Dr. Peehl were assessed and compared to those from snap-frozen biopsy samples. As shown in figure 2, the prostate TSCs demonstrated steady-state glycolytic and phospholipid metabolism, and bioenergetics that recapitulate features of prostate cancer *in vivo*. The TSCs were then applied to a perfused TSC bioreactor platform, and the bioenergetics (**Fig. 3**) and the dynamic pyruvate flux (**Fig.4**) of the TSCs were investigated by ^{31}P and HP ^{13}C MR, respectively. ^{13}C spectra following injection of HP ^{13}C pyruvate showed significantly increased pyruvate to lactate flux in malignant as compared to the benign prostate TSCs. This increased flux in the malignant prostate TSCs correlated with both increased expression of monocarboxylate transporters (MCT) and activity of lactate dehydrogenase (LDH) (**Figure 3**).
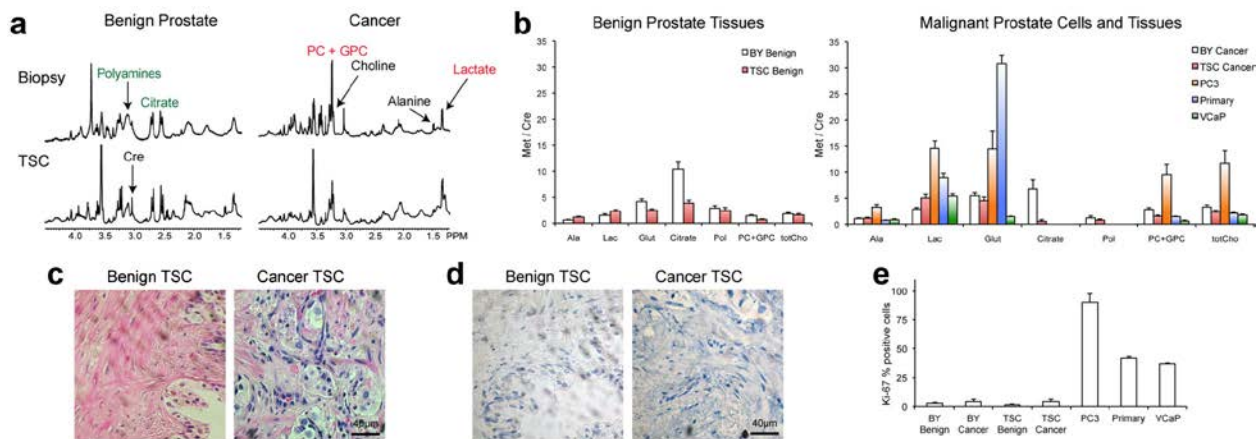
Fig. 2. a: Representative 1H HR-MAS spectra from benign and malignant snap-frozen human biopsies and TSCs post-2 hr of culture. b: Quantifiedmetabolite ratios for benignbiopsies and TSCs (N . 16 and14, respectively) as well as malignant BY and TSC (N = 12 and 9, respectively) compared to PC-3, primary prostate cancer cells and VCaP cells (all N . 4). c: Representative hematoxylin- and eosin- (H&E) staining and (d) Ki-67 immunohistochemistry of TSCs. e: Average percentpositiveKi-67 staining of TSCs as compared to the other prostate tissues and cells.Ala, alanine;Lac, lactate;Glut,glutamate;Cre, creatine;Pol,polyamines;PC . GPC, combined phosphocholine andglycerophosphocholine; Tot Cho, totalcholine. All ratios reported as mean ± SE.
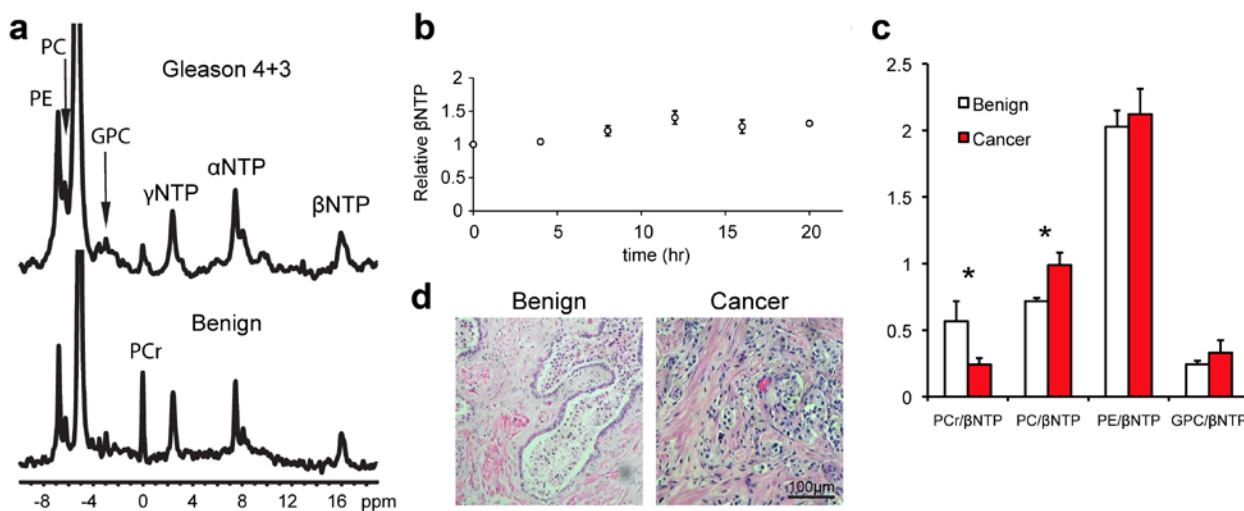
Fig. 3. a: ^{31}P spectra from living malignant and benign TSCs perfused in the MR-compatiblebioreactor. b:Timecourse of β-NTP demonstrating preservation of ATP in the bioreactor. c: Ratios of metabolites to β-NTP in benign (N = 4) and malignant TSCs (N = 4). d: Representative H&E staining of TSCs post-perfusion in the bioreactor. PCr, phosphocreatine; PC, phosphocholine; PE, phosphoethanolamine;GPC, glycerophosphocholine;
α,β,γ-NTPs, nucleotide triphosphates. All ratios reported as mean ± SE. P < 0.05.

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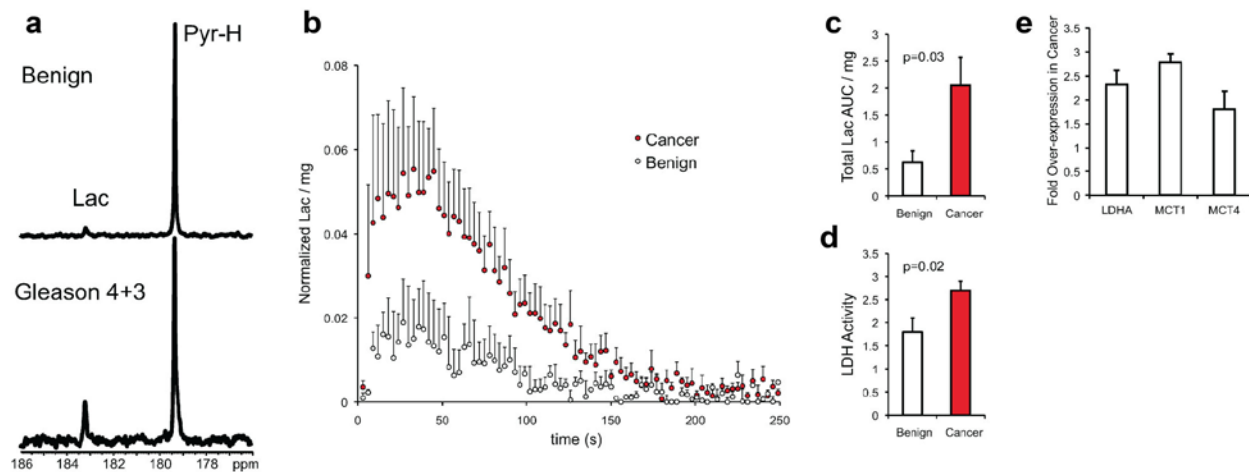
Fig. 4. a: A single spectrum taken at 90 sec post-injection of HP [1-^{13}C] pyruvate in the bioreactor with benign (N = 4) or malignant TSCs (N = 4). b: Average HP Lactate dynamics for TSCs. c: Total normalized lactate area under the curve (AUC) and (d) LDH activity for benign And malignant TSCs. e: mRNA expression of lactate dehydrogenaseA (LDHA), monocarboxylate transporters1 (MCT1) and 4 (MCT4). All plots are represented as mean ± SE.

Aim 3 was to identify hyperpolarized metabolic biomarkers of response to hormone and chemotherapy. As described in Dr. Peehl's progress report, we found that increasing the concentration of androgen (R1881) from 10 to 50 nM extended the longevity of benign and malignant TSCs from 1 to 5 days (Maund et al., see Reportable Outcomes). My group also demonstrated that reducing the concentration of androgen (R1881) to castrate levels (≤ 1.7 nM) in the media of the TSC bioreactor resulted in a dramatic reduction pyruvate to lactate flux within 24 hours of androgen reduction, whereas, a reduction in tissue ATP was not observed until 48 hours. The ATP reduction correlated with cell death as evidenced by live dead histology assays (LIVE/DEAD ® Viability/Cytotoxicity Kit) performed by Dr. Ronen's lab. The early reduction in pyruvate to lactate flux and subsequent reduction in ATP and cell viability appeared to be most dramatic for benign tissues and decreased with increasing cancer grade, however, more studies are necessary for statistical comparisons.

KEY RESEARCH ACCOMPLISHMENTS:

- We have engineered a robust MR compatible human prostate tissue slice culture bioreactor and used it to investigate prostate metabolism using standard ^{13}C and ^{31}P NMR and HP ^{13}C pyruvate MR.

- We have optimized the bioreactor set-up and data acquisition protocol.

- We have demonstrated that the steady-state metabolite concentrations in freshly excised human prostate TSCs demonstrated steady-state glycolytic and phospholipid metabolism, and bioenergetics that recapitulate features of prostate cancer *in vivo*.

- We demonstrated that the ^{13}C spectra acquired following injection of HP ^{13}C pyruvate showed significantly increased pyruvate to lactate flux in malignant as compared to the benign prostate TSCs. This increased flux in the malignant prostate TSCs correlated with both increased expression of monocarboxylate transporters (MCT) and activity of lactate dehydrogenase (LDH).

- We demonstrated that reducing the concentration of androgen (R1881) to castrate levels in the media of the TSC bioreactor resulted in a dramatic reduction pyruvate to lactate flux within 24 hours of androgen reduction, and a reduction in tissue ATP tissue viability at 48 hours. Whereas increasing the concentration of androgen (R1881) from 10 to 50 nM in the media extended the longevity of benign and malignant TSCs from 1 to 5 days in the bioreactor.

REPORTABLE OUTCOMES:

- Keshari KR, Sriram R, Van Criekinge M, Wilson DM, Wang ZJ, Vigneron DB, Peehl DM, & Kurhanewicz J Metabolic reprogramming and validation of hyperpolarized (13) C lactate as a prostate cancer biomarker using a human prostate tissue slice culture bioreactor. Prostate. 2013; 73(11), 1171-1181.

- Maund, S.L., Nolley, R. and Peehl, D.M. Optimization and comprehensive characterization of a faithful tissue culture model of the normal and malignant human prostate. Submitted to Lab Invest.

- Ronen, S.M. and Kurhanewicz, J. "Optimization and Characterization of a 3D human Prostate Tissue Culture Bioreactor" to be submitted to the Journal Magnetic Resonance in Medicine.

CONCLUSIONS: We have engineered a robust MR compatible 3D human prostate tissue slice culture bioreactor and used it to investigate prostate metabolism using standard 13C and 31P NMR and HP 13C pyruvate MR. Using this platform, we provided the first mechanistic evidence for HP ^{13}C lactate as a prostate cancer biomarker in living human tissues, critical for the interpretation of *in vivo* studies. More broadly, the clinically relevant metabolic model system, living human tissue culture bioreactor, in combination with HP MR can facilitate the identification of clinically translatable biomarkers of prostate cancer presence, aggressiveness, and treatment response.